STRUCTURAL COMPARATION OF CEREAL AND TUBER AMYLOPECTINS

by

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B.A. National University of Colombia,1973.

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Grain Science and Industry

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1985

Approved by

Major Professor

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Starch constitutes the major food reserve of higher plants. It contitutes up to 70% of the cereal kernel (22) and up to 24% of fresh tubers (38) . Starch granules vary in size, shape molecular structure and properties depending upon the botanical source (38). Starch performs two main roles in food products, it is a dietary carbohaydrate source (i,e nutritive) and it provides the characteristic viscosity,texture, mouth-feel and consistancy of many food products, both cereal based and non-cereal based. Starch also is freguently used as a processing aid to facilitate the manufacture of food products. As a consequence of its importance, starch, its structural and funtional properties has been the subject of a great deal of research over the last 60 years.

In order to understand the role of the starch ,as it relates to the quality of the final food products,it is necessary to know the differences of the intrinsic structure of the molecular components of starch as they exist from different starch sources . This study was undertaken to compare the fine structure of amylopectins from millet (Pennesetum americanum) wheat (Tritricum aestvium) mandioc (Manihot esculenta crantz) and ocumo (Xanthosoma sacittifolium).

LITERATURE REVIEW

Starch is an organized polymer composed of repeating D-glucose units.In nature it exist as discrete collections of molecules or granules. Chemical and biochemical evidence indicate that two kinds of carbohydrate polymers are present in the starch granule, amylose and amylopectin. They exist in different proportions according to the source of starch. The amylopectin fraction predominates in tubers and most of cereal starches .Significant differences in molecular structure and physical properties exist between these two types of polymers. The application of enzymatic methods and chromatographic techniques to the structural analysis of amylaceous compounds has been useful in elucidating the molecular structure of these polymers (2,8,9,15,21).

AMYLYTIC ENZYMES.

The enzymes which attack and degrade starch can be divided into two main groups; debranching enzymes, those that specifically hydrolyze the alpha 1-6 interchain linkages of amylopectin, and the alpha 1-4 glucosidases, those that split the alpha 1-4 glycosidic linkages. The later can be further subclassified into enzymes that make random or internal clevages (endo acting enzymes) and those that act sequentilly from chain ends (exo acting enzymes) (27).

Among the enzymes which attach starch ALPHA-AMYLASE is specific for alpha 1-4 glycosidic linkages.(26) Its action pattern is essentially random and directed to form primarily alpha- limit dextrins and some D-glucose and maltose .The former are branched oligosacharides of dp 4 or more, containing alpha 1-6 D-glucosidic linkages (15,26).

B-AMYLASE (E.C.3.2.1.2.), on the other hand, is an exo-enzyme starting at the non-reducing end of the polymer, it splits alternate alpha 1-4 bonds in amylaceous polysaccharides yielding maltose. It can not split alpha 1-6 glycosidic linkages of amylopectin neither can it pass alpha 1-6 branch points.The A or outer chains of amylopectin are degraded by B-amylase to a predictable length of two or three glucose units depending on whether the chain contained and odd or even numbers of glucose residues before digestion (19,35).Thus,an odd number will result in two residues remaining while an even number in three residues remaining. When amylopectin is treated with B-amylase approximately 50% of the carbohydrates are converted into maltose (26,27).The remaining B-amylose resistent polymer is referred to as a B-limit dextrin.

Enzymes that specifically hydrolyse alpha 1-6 glycosidic bonds have great utility in the structural analysis of starch. PULLULANASE (E.C.3.2.1.41) first isolated in 1961 (35), acts on

amylopectin to affect nearly complete removal of its branches (1,28) by its action on alpha 1-6 bonds.The endo enzyme also debranches B-limit dextrins, releasing maltose and maltotriose from the original A chains and higher oligosaccharides (presumably from the B chains) (26). Pullulanase debranching actin on amylopectin, glycogen, and their B limit dextrins, followed by chromatographic fractionation of the resulting carbohydrate polymers has been used in the determination of the average of chain length ($\overline{CL}$) of these polysaccharides and their constituent branches (2,8,9,10,19,24,25).

Harada et al (20) and Whelan et al (18) isolated and characterized a second enzyme,ISOAMYLASE,which can be used in structural determinations. The specificity of isoamylase is such that it completely debranches glycogen and amylopectin as well as the phosphorylase limit dextrins of these polysaccharides.(26) Its action on the B-limit dextrins of glycogen and amylopectin is incomplete (10,18).Specifically, isoamylase is able to cleave only alpha 1-6 linkages present at branch points in oligo and polysaccharides, and requires an outer or A chain of three or more glucose units before debranching can occur (27).

AMYLOSE STRUCTURE.

Enzymatic and physical studies have indicated that the

amylose component of starch in not a simple linear alpha 1-4 glucoside. (8,32) .Pure B-amylase preparations result in only approximately 70% conversion of amylose to maltose This has led to the conclusion that amylose consists of essentially linear polysaccharides and some what larger polysaccharides containing barriers to enzyme action (4,5) , probably some low levels of branching. Of course,an alternate explanation for the incomplete conversion of amylose to maltose by B-amylase is the presence of contaminanting amylopectin or artefactual barriers to enzyme created during the polysaccharide solation procedure (26).

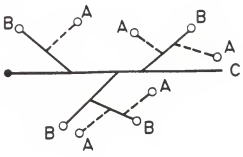
AMYLOPECTIN STRUCTURE.

In the period before it became possible to explore the fine structure of amylopectin enzymatically, several structures for amylopectin were proposed that equally satisfied the known facts about the molecule. Early chemical methods showed that, although glucose units in amylopectin were linked primarily through alpha 1-4 glycosic bonds branching occurred through alpha 1-6 glycosidic linkages. his fact, the presence of one alpha 1-6 linkage in about twenty-four glucose units results in an infinitely large number of possible arrangements.

Peat and coworkers (38) divided the carbohydrate chains in the amylopectin molecule into three types,A,B,and C chains.

A-chains are those being no substituent chains or branch

points. Thus,they are linked to the rest of molecule only through their reducing ends.

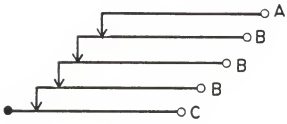


o terminal non reducing group

● reducing group

B-chains are those that are linked to the molecule through their reducing ends but, in addition are branched at the C-6 position in one or more of their B-glucopyranosyl residues.The C-chain is the only carbohydrate chain in the molecule having a free reducing group.

The structures proposed by Haworth et al (38), Standinger et al (35) and ,Meyer (35) differ primarily in the proportions of A and B chains. In the Haworth structure, in which the chains carry only one substituent chain, there is only a single

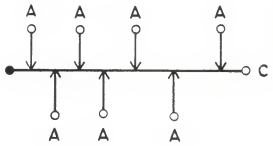


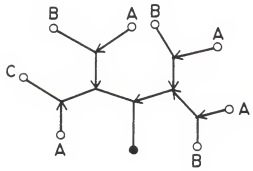
LAMINATE STRUCTURE:

of Harword et al.

HERRINGBONE MODEL:

of Staudinger et al.





RANDOMLY BRANCHED MODEL:

of Meyer et al.

A chain and a single C chain, the rest of the molecule is composed of B chains. Standinger's structure, on the other hand, has all its chains with the exception of the C chain, unsusbstituted. A chains branch out exclusively from the C chain with the result that there are no B-chains at all. Meyer's model has a randomly branched structure with equal numbers of A and B chains.

To distinguish between these three structures, enzymatic digestion followed by gel filtration has been used to directly measure the numbers of chains of each type following debranching, the average chain length (Cl^*) of the released polymers can be determined (19,26). These values vary according to the amylopectin source. A Cl range of 17 to 20 has been reported for wheat amylopectin (24) and values of 25 for mandioc amylopectin (21).

An important parameter used to characterize the degree of multiple branching in the amylopectin molecule is the ratio of A to B chains (A:B ratio) .By using isoamylase or pullulanase on B-limit dextrin that ratio can be calculated (25).Incubation with isoamylase alone results in incomplete debranching of amylopectin as a consequence of the inability of this enzyme to hydrolyse A chains that are two glucose units or less. This constituterougly half of the total numbers of chains. Amylopectin typically contains approximately twice as many A chains as B chains. A:B chain ratios are markedly higher in

amylopectin (1.5-2.6:1) than they are in glycogen (0.6-1.2:1) (27). Lii et al. (24) reported A:B chain ratios for different types of wheat amylopectin ranging from 1.21 to 2.11.

More recently gel-filtration chromatography of the low molecular weight debranched products of amylopectin, used to obtain a chain size profile has shown the existence of a bimodal distribution of carbohydrate polymers in amylopectin (17,21,24,27,29,33). This leads to the conclusion that amylopectin structure can be correctly described by the revised Meyer model proposed by Gunja-Smith et al. and furter by the cluster models of French 1972 (15) and Robin et al 1974 (33).Wheather the bimodal distribution results from the same polysaccharide molecules or not has been studied by different investigators (2,9,26). Marshall et al (27) suggested that the chain populations come from two different populations of polysaccharides. Atwell et al (2), by studying the B- limit dextrins of wheat amylopectin found that the overall amylopectin population was uniform and concluded that the bimodal distribution of chain sizes came from two chain populations or classes that existed within the same macromolecule.

MATERIALS AND METHODS

A.- MATERIALS.

1.-STARCHES.

WHEAT:

Hard wheat flour was obtained from the pilot scale mill at Kansas State University. Wheat starch was isolated by the dough kneading procedure (39). Dough balls were kneaded under runnig water over 20xx bolting cloth and the aqueous slurry obtained centrifugated at 2000 xg for 30 minutes. The tailings layer was removed by scraping with spatula and the prime starch resuspended in water and centrifuged until no tailing layers was obtained.The recovered prime starch was lyophylized.

PEARL MILLET:

To obtain millet starch, pearl millet grain was soaked at 4^{o} C for 24 hours in distilled water containing 0.01% sodium azide.It was then washed several times with distilled water . The wet millet grain was ground in a Waring blender for 3 minutes,and screened through a 112 xx bolting cloth. The material retained on the screen was ground again for one minute and passed again through the bolting cloth. The slurry

obtained was allowed to settle and the supernatant was removed. the sediment was reslurried and passed through a 20 xx screen .The aqueous slurry obtained was centrifuged at 2000xg for 30 minutes and the tailings layer was removed by scraping it with a spatula.The resulting prime starch was reslurried in water and centrifuged until no tailing layer was obtained.The recovered prime starch was lyophylized.

MANDIOC AND OCUMO :

Starches from mandioc (Manihot esculenta crantz) and ocumo (Xanthosoma-sacittifolium) were the generous gift of Dr. E.Schultz of the Central University of Venezuela, Maracay,Venezuela. These tuber starches were obtained by wet milling and dried in a forced air oven at 45 ^{o}C. In addition, mandioc starch obtained from Colombia,was produced my mother, Rosa Gutierrez. It was made by a household procedure. Fresh mandioc was peeled and scraped. It was suspended in water and screened through a bolting cloth and the aqueous slurry was allowed settle. After decanting the supernatant the tailings layer was removed by scraping. The procedure was repeated several times until no more tailings layer was present.The prime starch was dried in the open air for three days.

B.- METHODS.

STARCH FRACTIONATION:

Prime starches were fractionated into amylose and amylopectin fractions by the extraction- sedimentation procedure of Montgomery (30). Two liters of 2% (w/v) starch in phosphate buffer, (pH 6.0-6.3) was heated at 95-98 ^{o}C for 11 minutes befored autoclaving (20 psi) for two hours.The solution was saturated with n- pentanol (500 ml) and allowed to cool overnight with gentle stirring.The amylose - pentanol complex was collected by centrifugation (3000xg for 20 minutes)." Pure" amylose was obtained by adding 1.5 l. of hot water that had been saturated with n-butanol (100 mls) to the amylose and stirring overnight. The precipitat amylose complex was recovered by centrifugation (3000 xg for 20 minutes).The above procedure was repeated three times for mandioc and ocumo samples and five times for wheat and millet samples. Recovered purified amylose was mixed with fresh n- pentanol in a Waring blender and filtered thru a coarse porosity,scintered glass funnel before being lyophylized.

Amylopectin was recovered from the original supernatant by adding an equal volume of methanol to the aqueous solution. The amylopectin was allowed to precipitate overnight under refrigeration. The

amylopectin,then,was mixed with methanol in a waring blendor (15 sec) and filtered thru a coarse porosity scientered glass funnel before being lypholyzed.

IODINE AFFINITY.

The iodine affinity of starches,and its fractions amylose, and amylopectin were determined by potentiometric titration (34). Starches, amylose, and amylopectin fractions from millet and wheat were defatted by soxhlet extraction for 24 hours with 95% ethanol.The samples were then lyophilized and pulverized before the determination.A Corning pH-meter (Model 140) with a calomel electrode as reference and a bright platinum electrode was used for analysis. Two minutes was allowed for equilibration between the addition of iodine solution and obtaining a reading. Since deionized water was found to be essential for reproducible results, it was used for all determinations. Results are report as percent og iodine bound.

AMYLOSE CONTENT:

The amylose content of starches, amylose, and amylopectin fractions was determined by the colorimetric procedure of Bienvenido (7) using pure amylose and pure amylopectin from potatoe as standards. Spectrophotometric readings at 620 mm were takeng with a Beckman spectronic 21

(250 Harbor Blvd.,Fullerton,C.A. US. 92634). All determinations were run at least four times. Results are report as percent of amylose.

PREPARATION OF B-LIMIT DEXTRIN.

Amylopectin (1g) was dissolved in 0.1 M NaOH (30 ml) in a 50 ml volumetric flask .The pH was adjusted to approximatly 6 with 1M HCl. Five ml of 0.2 M acetate buffer (pH 4.8) was added and the sample diluted to volume. An aliquot (1 ml) diluted 1:100 was used to determine total carbohydrate in the samples by the phenol - sulfuric acid procedure (14). The determination was run 5 times for each sample. B-amylase (4500 units, type 1-B lot 122F-8120, Sigma Chemical Co, St Louis, Mo.) was added to the flask and the total solution dialyzed against repeated changes of 0.02M acetate buffer (pH 4.8) to remove carbohydtrate,generated by the enzyme action. Several drops of toluene were added to the dialysis bag to inhibit microbial growth.After 24 hours an additional 1000 units of B- amylase were added and the dialysis continued until no carbohydrate was detected in the dialyzate.The contents of the dialysis bag were concentrated, transfered to a 50 ml volumetric flask and diluted to volume. An aliquot (1 ml) diluited 1:100 was used to determine total carbohydrate (see above) and the results compared with the original

solution to obtain the B-amylolysis limit. The remainder of the solution was heated for 15 minutes in a boiling water bath , filtered through Whatman # 42 filter paper and lyophylised.(24,25,36).

DEBRANCHING WITH PULLULANSE:

Pullulanse from Eterobacter aerogenes (lot 122F-8120 and 14F 02031) was obtained from Sigma Chemical Co.St Louis,Mo. as a suspension in 3.2 M $(NH_4)_2SO_4$.It was used as received unless the Nelson's colorimetric copper procedure was to be subsequently performed (31).Since ammonium sulfate interferes with the reduction of copper in the assay it was removed by dialysis against water before any analysis of reducing power was done. The enzyme did not show any alpha (1-4) glucosidase activity (see appendix II.)

Amylopectin (100 mg) or B-limit dextrin (50 mg) was dissolved in 0.1M NAOH (5 mls) and neutralized with 1M acetic acid before dilution to 10 mls with acetate buffer (0.1 M,pH 5.5). Total debranching was obtained by adding 16 units of pullulanase to the solution and incubating at 37°C for 36 hours.The digests were subsequently heated in boiling water for 20 minutes to inactivate the enzyme.Insoluble material was removal by filtration.(2,9,24).

DEBRANCHING WITH ISOAMYLASE:

Isoamylase from Pseudomonas amyloderamosa (lot 73F-03581B) was obtained fron Sigma Chemical Co.St Louis Mo.Since it did not show contaminating alpha 1-4 glucosidase activity (see appendix III) it was used as received.

Amylopectin (50 mgs) was dissolved in 0.1 M NaOH (5mls) and neutralized with 1M acetic acid before dilution to 10 mls with acetate buffer (0.1 M, pH 5.5) . Total debranching was performed using 0.27 units of isoamylase and incubating at 37^{O} C for 24 hours . The digest was heated in boiling water for 5 minutes to inactivate the enzyme and insoluble material was removed by filtration (24).

GEL FILTRATION :

Solutions containing approximately 5 mgs of debranched amylopectin were chromatographed on pharmacia K 26/70 columns (2.5x75 cms) of Bio-gel P-10 or Bio-gel P-2. The columns were operated in the upflowmode.Carbohydrate was eluted with water containing 0.02% NaN_3 (2,9,24). The flow rate was 10 to 15 ml per hour.Fractions of approximately 4 mls were automatically collected with a Golden Retriever, Fraction collector, model 1200 pup

(Instrumentation Specialties Company, Inc. Lincln,Nebr.68504.U.S.A.) using the drop counting mode. Carbohydrate in the effluent fractions was quantitated by assaying an aliquot of each fraction using the phenol-sulfuric acid procedure (14). Each column was calibrated using 80 mgrs of pullulanase debranched waxy corn starch. The DP of the resulting fractions was obtained by calculating the total amount of carbohydrate in each fraction over the amount of reducing power in that fraction. Occasionally it was necessary to combine three or more fractions and concentrate to obtaine sufficient material for the determination.

RESULTS AND DISCUSSION

CHARACTERIZATION OF STARCHES AND THEIR FRACTIONS:

The proximate analyses of starches from millet (Pennesetum americanum),wheat (Tritricum aestivium),Venezuelan mandioc and Colombian mandioc(Manihot esculenta crantz) and ocumo (Xanthosoma sacittifolium), are shown in table I .Their isolated amylose and amylopectin fractions are shown in the table II and III respectively. As measured by potentiometric titration (34) the iodine affinity values (table II) for whole starches, ranged from 3.01 for Venezuelan mandioc starch to 4.89 for millet starch.These values agree well with those reported for wheat (24) millet (6) Mandioc (37). The iodine affinity of ocumo starch has not been reported previusly . The value obtained for this starch (4.06) is higher than those previosly reported for tuber starches (37) .The amylose content for these starches is reported in tableII.Values ranged from 15% for Colombian mandioc starch to 23% for wheat starch.These values agree well with those reported for wheat (37) millet (3,6) and mandioc (11).The amylose content of ocumo starch has not been reported.The results here (22%) suggest that ocumo starch contains a greater amount of amylose than do other tuber starches.

The isolated amylose, obtained by Montgomery's procedure,(30)

had iodine affinities ranging from 17.91% for Colombian mandioc to 83% for millet. Iodine affinity values for mandioc amylose samples agree well with those reported before (11). Defatted wheat and millet amyloses, on the other hand,showed iodine afinities some what lower than those reported previosly (2,6,24,26,),although they were recrystalized five times. Similar results were obtained by measuring amylose content in these starch samples samples by a second method (7) (tableIII). The tuber amylose samples (mandioc and ocumo) were purer (84-90%) that the amylose samples isolate from cereals (wheat and millet) (75%-79%). This results showed that probably the structure of these cereal amylose fractions are differnt fron that of tuber sample used as standard . These structural differences may have resulted in the purity of these cereal amylopectin fractions to be understimated.

CHARACTERIZATION OF THE ISOLATED AMYLOPECTINS.

The isolated amylopectins from all five sources were quite pure as judged by their iodine affinity values and amylose contents (tables II and III), since the values agreed well with those reported by previous workers (2,3,4,6,11,24,26,). The samples were pure enough to use for subsequent debranching and chromatographic studies.

Incubation of amylopectin samples with pullulanase

(E.C.3.2.1.41) or isoamylase (E.C. 3.2.68) followed by gel filtration, on a Bio-gel P-10 column, yielded a bimodal distribution of carbohaydrate chains from all the samples (Fig 1-5). Elution profiles of all the amylopectin samples appeared to be similar with slight differences in the DP of their carbohydrate populations (Table IV). The leading peak, with largest Mw material, showed several differences.between samples It contained material with an average degree of polymerization (DP) of 50-58 for debranched wheat and ocumo amylopectins.The DP of the material in this fraction was larger than 58 for debranched Venezuelan and Colombian mandioc amylopectins as well as for debranched millet amylopectin.The second,later, elutin peak contained material with an average degree of polymerization $\overline{DP}$ of 10-16 for debranched wheat and ocumo amylopectins (table IV). The DP of the same peak for debranched amylopectin from millet and mandioc was larger (18-22). These results agree with previous studies on mandioc(21) wheat (2,24). The presence of these two carbohydrate populations leads to the conclusion that the amylopectin fractions for the samples studied here can be best described by the symmetric model proposed by Gunja-Smith et al (31) as well as the cluster model proposed by French (29).

The third, minor,peak observed in the chromatographs at the void volume can be explained as the presence of amylose contamination in the amylopectin (2,9,24). Incubation with the

Fig. 1.- Bio- gel P-10 .Elution profiles of millet amylopectin (———). Pullulanase debranched millet amylopectin (- - -) . Isoamylase debranched millet amylopectin (-·-·-·).

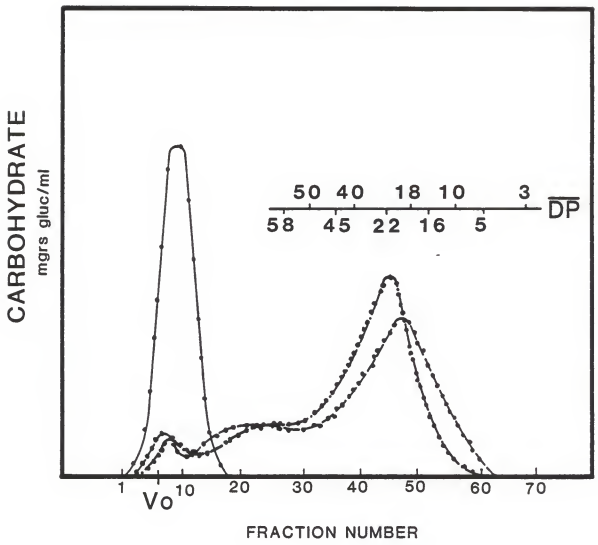

CARBOHYDRATE
mgrs gluc/ml
50 40 18 10 3
58 45 22 16 5
DP
1
Vo
10
20
30
40
50
60
70
FRACTION NUMBER

Fig.2.- Bio-gel P-10. Elution profile of wheat amylopectin (———) Pullulanse debranched wheat amylopectin (----) Isoamylase debranched wheat amylopectin (-·-·-).

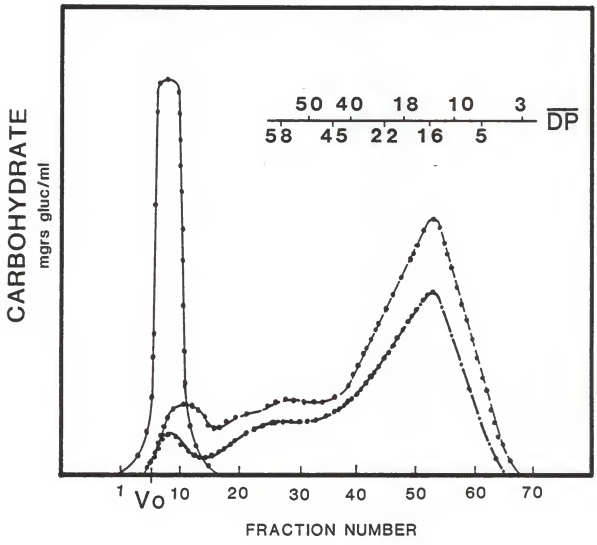

CARBOHYDRATE
mgrs gluc/ml
50 40 18 10 3
58 45 22 16 5
DP
1 Vo 10 20 30 40 50 60 70
FRACTION NUMBER

Fig. 3.- Bio-gel P-10 . Elution profiles of Venezuelan mandioc amylopectin (——). Pullulanase debranched Venezuelan mandioc amylopectin (– – – –). Isoamylase debramched Venezuelan mandioc amylopectin (–·–·–·–).

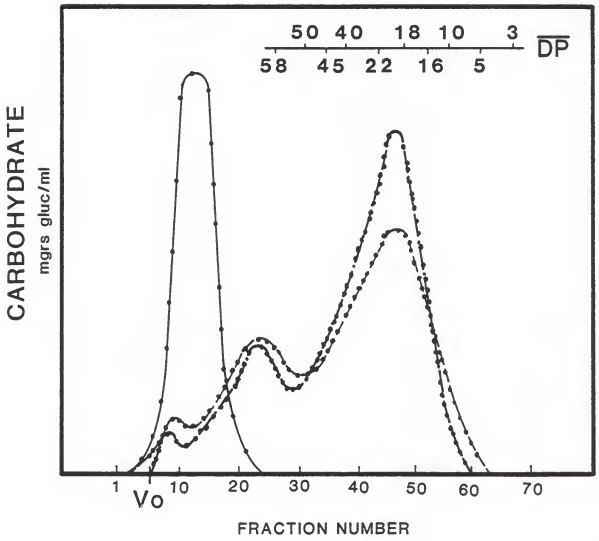

50 40 18 10 3
58 45 22 16 5
DP
CARBOHYDRATE
mgrs gluc/ml
1
Vo
10
20
30
40
50
60
70
FRACTION NUMBER

Fig. 4.- Bio-gel P-10 . Elution profiles of Colombian mandioc amylopectin(———). Pullulanase debranched Colombian mandioc amylopectin (– – –) Isoamylase debranched Colombia mandioc amylopectin (–·–·–·)

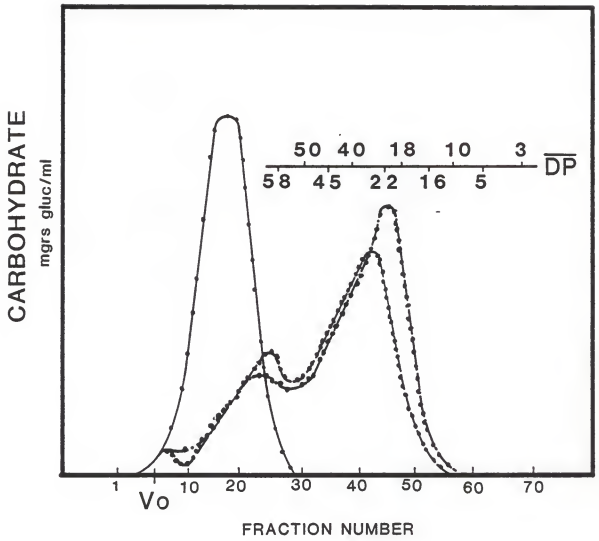
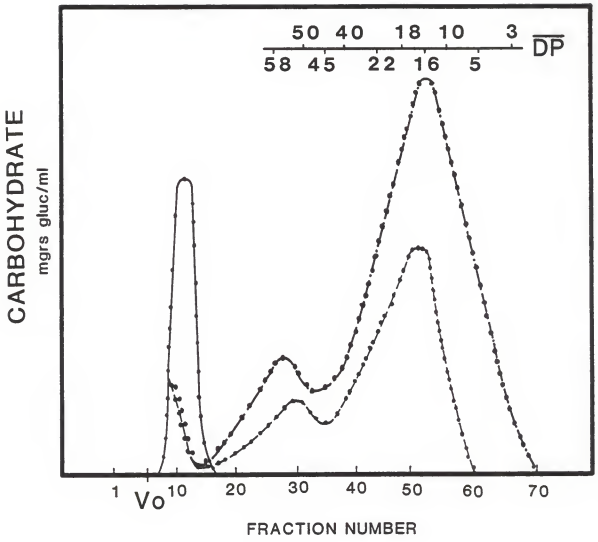

CARBOHYDRATE
mgrs gluc/ml
50 40 18 10 3
DP
58 45 22 16 5
1
Vo
10
20
30
40
50
60
70
FRACTION NUMBER

Fig. 5.- Bio-gel P-10 . Elution profiles of ocumo amylopectin (———). Pullulanase debranched ocumo amylopectin (----). isoamylase debranched ocumo amylopectin (— · — · —).


50 40 18 10 3
58 45 22 16 5
DP
CARBOHYDRATE
mgrs gluc/ml
1 Vo 10 20 30 40 50 60 70
FRACTION NUMBER

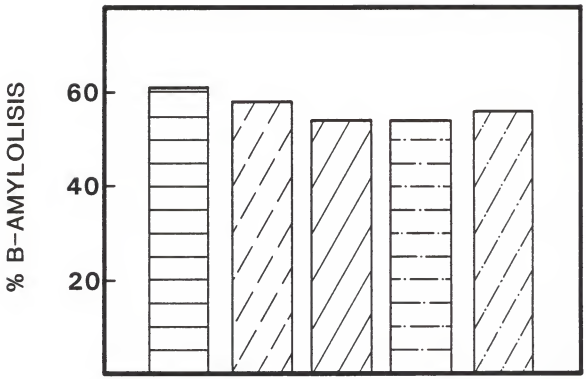
enzymes was done until no changes in that peak as detected.

The average of the chain length of the debranched amylopectins, obtained from the total amount of the polysaccharide debranched by pullulanase divided by the amount of reducing power liberated during debranching, (19) (table IV) revealed that Venezuelan mandioc amylopectin has the largest $\overline{Cl}$, 24, while ocumo amylopectin has the smollest $\overline{Cl}$, 16.The average chain length of wheat and mandioc amylopectins are comparable to those reported by previous workers (21,23,24). Cl of millet and ocumo amylopectins have not been previously reported. Our data suggests that the average chain length for millet amylopectin (Cl 17) was similar to that obtained for wheat amylopectin. The $\overline{Cl}$ of ocumo, (16),on the other hand, was lower than that of mandioc amylopectins, (24-22) .Thus ocumo amylopectin has both more and shorter chains than does mandioc amylopectin (25).

Incubation of amylopectin samples with B- amylase produced B- amylolysis values ranging from 54% for Venezuelan and Colombian mandiocs to 61% for millet (Fig.6) . B-amylolysis limits for wheat and millet amylopectins are higher than that for Venezuelan and Colombian mandiocs and ocumo amylopectins.This may well reflect structural differences in the molecule.Thus, wheat and millet amylopectin molecules may be more branched than are mandioc and ocumo amylopectins .

After removal of maltose and by dialysis the amylopectin

Fig. 6.- Per cent B- amylolysis of amylopectins from: Millet (═══). Wheat (,,,,,). Venezuelan mandioc (///). Colombian mandioc (-·-·-). Ocumo (,,,,,).


% B-AMYLOLISIS
60
40
20

B- limit dextrins were debranched by pullulanse, chromatographied on Bio-gel P-2. Their elution profiles showed a similar distribution of carbohydrates in all the samples with only slight diferences in average chain lengths. Three main populations can be distinguished (fig 7-8). The highest Mw material consisted of long linear chains of $\overline{DP}$ 37-50 .The intermediate Mw material contained linear chains with $\overline{DP}$ of 5-37 .Those populations correspond to the B-chains.The smallest Mw population contained maltose and maltotriose as the result of the release of fragments of debranched A chains. These patterns corroborated the profiles obtained from pullulanase debranched starch-limit dextrin of waxy maize (23) . The A:B chain ratio can be calculated by measuring the amount of reducing groups librated from A chains after debranching (8) as a fraction of the reducing groups liberated from B chains during debraching (the rest of population).When this was done (table V) it shown that millet amylopectin has the largest A:B chain ratio (1.79) (table V) while Venezuelan mandioc amylopectin, the shortest (1.42). Because A chains are unbranched and B chains are those which have another chain attached, the larger A:B ratio for millet amylopectin indicates that its amylopectin has a greater extent of multiple branching than the do other samples. This is particularly true relative to the Venezuela mandioc amylopectin. Colombian mandioc amylpectin apparently has is less multiple branched than are

Fig. 7.- Bio gel P-2 . Elution profiles of pullulanase debranched of millet B- limit dextrin (———) .Wheat B-limit dextrin (----).

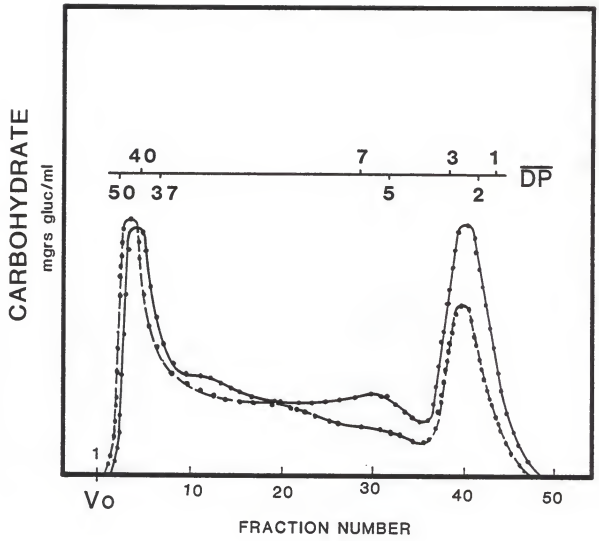
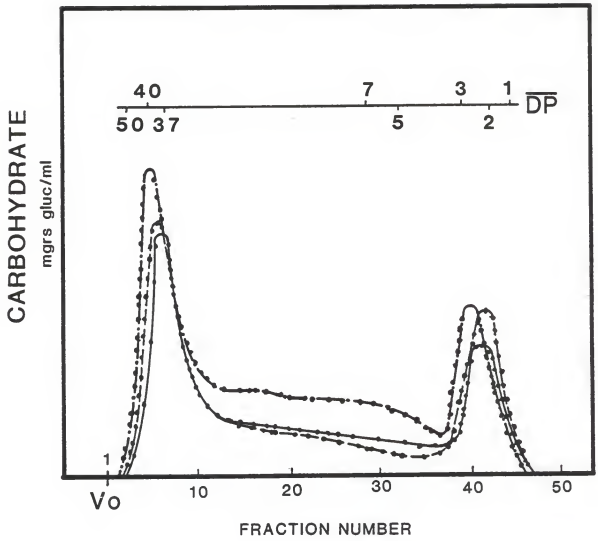

CARBOHYDRATE
mgrs gluc/ml
40
7
3
1
DP
50
37
5
2
1
Vo
10
20
30
40
50
FRACTION NUMBER

Fig. 8.- Bio-gel P-2. Elution profile of pullulanase debranchin of Venezuelan mandioc B-limit dextrin (———) Colombian mandioc B- limit dextrin amylopectin (----) Ocumo B- limit dextrin (—·—·).


CARBOHYDRATE
mgrs gluc/ml
40
50 37
7
5
3
2
1
DP
1
Vo
10
20
30
40
50
FRACTION NUMBER

wheat or ocumo amylopectins.

For the amylopectin fractions studied the average chain length ; the B- amylolysis limits ;and the A:B chain ratio were internally consistent and supported these conclusion . Millet, for example , has the lowest average chain length (17) ,the highest B- amylolysis limit (61%) and thehighest A:B chain ratio (1.79) as expected for a more highly branched molecule. Manioc, on the other hand, has the highest average chain length (22-24), the lowest B- amylolysis limit (54%), and the lowest A:B chain ratio (1.42) again consistent and supporting a less highly branched molecule. These results led to the conclusion that millet, wheat, and ocumo amylopectin fractions possess more and shorter branches than do mandioc amylopectin fractions.

Thus, the fine structure of these amylopectins apparently have definite but minor diferences in their structure.

SUMMARY AND CONCLUSIONS

Starch isolated from millet (Pennesetum americanum) wheat (Tritricum aestivium) Venezuelan and Colombian mandioc (Manihot esculenta crantz) and ocumo (Xanthosoma sacittifolium) were fractionated into their amylose and amylopectin components.The fine structures of the isolated amylopectins and its B-Limit dextrins were investigated using hydrolytic enzymes in conjuntion with gel-filtration chromatography . The isolated amylopectin fractions treated with B-amylase appear to have B-amylolysis limits ranging from 54% for Venezuelan and Colombian mandioc amylopectin fractions to 61% for millet amylopectin fraction.

When the amylopectin fractions were debranched with pullulanase or isoamylase,the debranched fractions had unit chain profiles very similar to each other with two chains populations DP of approximately 10-16 and 50-58 respectively in wheat and ocumo amylopectins and DPs of 18-22 and > 58 respectively in millet, and Venezuelan and Colombian mandioc amylopectins.

The average chain length of the amylopectin ranged from 15 for ocumo amylopectin to 24 for Venezuelan mandioc amylopectin. Ocumo amylopectin appears to be composed of more and shorter chains than for other tubers .

The ratio of A:B chains in each amylopectin fraction, determined enzymatically ranged from 1.42 for Venezuelan mandioc amylopectin to 1.78 for millet amylopectin.

The data obtained for B-amylolysis, Cl^{*} debranched amylopectin and A:B ratio chain lenght were internally consistent and leads to the conclusion that millet, wheat, and ocumo amylopectin fractions has more and shorter branches that dose mandioc amylopectin fractin .

In conclusion the amylopectins investigated here appear to have definite, but minor differences in structure.

TABLE I

Proximate analysis of starch samples.

Starch-Samples	% Moisture	% Protein (Nx5.7) (dry basis)	%.Ash (dry basis)
Millet	5.0	0.68	0.15
Wheat	7.4	0.43	0.17
Venezuelan mandioc	11.6	0.17	0.18
Colombian mandioc	14.7	0.35	0.10
Ocumo	9.5	0.22	0.08

TABLE II

Iodine affinity of starch samples and their fractions.

Source	% of Iodine bound		
	Starches	Amyloses	Amylopectins
Millet	4.89 ±0.23	18.83 ±0.5	0.93 ±0.03
Wheat	4.56 ±0.35	17.95 ±0.32	1.08 ±0.09
Venezuelan mandioc	3.01 ±0.31	18.86 ±0.30	0.75 ±0.17
Colombian mandioc	3.08 ±0.44	17.91 ±0.15	0.35 ±0.05
Ocumo	4.06 ±0.20	18.06 ±0.14	1.35 ±0.13

TABLE III

Amylose content of starch samples and their fractions.

Source	amylose content %		
	Starches	Amyloses	Amylopectins
Millet	22±0.69	79±0.95	2.0±0.4
Wheat	23±1.2	75±1.41	1.7±0.2
Venezuelan mandioc	16±1.7	84±1.82	6.0±0.6
Colombian manioc	15±1.8	85±0.96	4.0±0.2
Ocumo	22±1.1	90±0.5	6.0±0.4

TABLE IV

Characteristic of the isolated amylopectins.

Source	$\overline{CL}$*	$\overline{DP}$**	
		I	II
Millet	17 ±1.5	>58	18-22
Wheat	17 ±1.5	50-58	10-16
Venezuelan mandioc	24 ±1.8	>58	18-22
Colombian mandioc	22 ±2.0	>58	18-22
Ocumo	16 ±1.5	50-58	10-16

* Average chain lenght of pullulanase debranched amylopectins.

** Average of degree of polymerization of chain population resulting from the chromatography of pullulanase debranched amylopectins.

TABLE V

A:B chain ratio of isolated amylopectins.

Source	A:B ratio
Millet	1.79±0.08
Wheat	1.54±0.06
Venezuelan mandioc	1.42±0.05
Colombian mandioc	1.50±0.06
Ocumo	1.64±0.05

ACKNOWLEDGEMENTS

The author express her gratitude to; Dr. J. Faubion, and Dr. R.C. Hoseney for their guidance and patience during the course of her work.

Also,she wants extended her thanks to; Dr. P. Nordin for serving on her graduate committe, Dr. D. Fung who made it possible for her to achieve a goal, and the entire staff of the Department of Grain Science and Industry who in one way or other have made possible the development of this work.

DEDICATION

To my family especially my mother,Rosa, and my son, Edgar Alejandro, I dedicate my work and my life for. Without the moral support and encouragement of them,who I love, accomplishment of my goals would not been possible.

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APPENDIX I

B-amylolysis of each amylopectin fraction was determined by comparison of the total amount of carbohaydrate present in the amylopectin solution before and after digestion with B-amylase .

$$\frac{\text{Total carbohydrate before - total carbohydrate after}}{\text{total carbohydrate before}}$$

Average of total carbohydrate determination before and after digestion with B-amylase was.

Samples	Total carbohydrate (gluc.mg/ml).	
	Before digestion $\overline{X}_b$	After digestion $\overline{X}_a$
Millet	4.98 $\pm$0.5	1.94 $\pm$0.05
Wheat	4.75 $\pm$0.4	1.97 $\pm$0.03
Venezuelan mandioc	5.10 $\pm$0.2	2.34 $\pm$0.03
Colombian mandioc	5.10 $\pm$0.2	2.33 $\pm$0.02
Ocumo	4.98 $\pm$0.2	2.18 $\pm$0.02

APPENDIX II

To test for the existence of alpha (1-4) glucosidase activity in pullulanase a maltotriose solution was treated with the enzyme at 37_{o} C for 24 hours.The reducing power of the solution was determined before and after the treatment. by Nelson'copper procedure .

Reducing power

gluc. mgs / ml.

Before	After
2.78	2.78
2.74	2.79
2.78	2.75
2.76	2.74
$\bar{X}$ = 2.76 $\pm$0.02	$\bar{X}$ = 2.76 $\pm$ 0.02

APPENDIX III

To evaluate the alpha (1-4) glucosidase contamination of isoamylase a maltoheptaose solution was treated with the enzyme at 37 °C for 24 hours. The reducing power of the solution was evaluated by Nelson'procedure before and after the treatment, each determination was done four times.

Reducing power

gluc.mgs /ml.

Before enzyme treatment	After enzyme treatment
3.54	3.54
3.55	3.55
3.58	3.59
3.54	4.00
$\overline{X}$= 3.55 $\pm$0.02	$\overline{X}$ = 3.67 $\pm$0.22

STRUCTURAL COMPARISON OF CEREAL AND TUBER AMYLOPECTINS

BY

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B.A., National University of Colombia,1973

AN ABSTRACT OF A MASTER THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Grain Science and Industry

KANSAS STATE UNIVERSITY

Manhattan ,Kansas.

1985

The fine structure of amylopectin fractions from millet, (Pennesetum americanum) wheat (Triticum aestiviun) mandioc (Manihot sculenta crazt) and ocumo (Xanthosoma sacittifolium) were investigated using hydrolytic enzymes (B- amylase, pullulanase, and isoamylase) in conjunction with gel filtration chromatography (bio- gel P-10 and P-2). The unit chain profiles of debranched amylopectin fractions were characterized by two populations with average degrees of polimerization of 10-16 and 50-58 respectively in wheat and ocumo and 18-22 and >58 in millet and mandioc. The average chain length for these amylopectin fractions (16-24) ,the B-amylolysis limits (54-61%) and the A:B chain ratios (1.42-1.79) were internally consistent and led to the conclusion that millet, wheat and ocumo amylopectins possess more and shorter branches that dose mandioc amylopectin. In conclusion the amylopectin fractions study here appear to have definite but minor differences in structure.